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Pooling of microbiological samples with ABL Diagnostics' DeepChek® assays & software and MGI's DNBSEQ-G400 sequencing platform

Highlights

Simultaneous Analysis

ABL Diagnostics' DeepChek® assays and software can be used on MGI DNBSEQ™ on a large variety of microbiology applications simultaneously.

Optimized Workflow

Established NGS workflow that incorporates a fully automated workflow and micro pooling, optimizing turnaround time and NGS sequencing costs.

Exceptional Quality

Sequence with exceptional read quality, mapping, and coverage uniformity.

- Effective rate: >99%,
- Q30: >95%,
- Duplication rate: <1.5

Introduction

Subtype and drug-resistance mutations have been mostly assessed routinely using Capillary Electrophoresis (CE) sequencing which does not detect co-infection or minor variants (frequency below 15-20%). Next Generation Sequencing (NGS) has become the new standard for genotypic drug resistance testing. Currently, MGI Tech Co. Ltd (MGI) offers a collection of DNBSEQ™ genetic sequencing platforms to support NGS, providing users with comprehensive, flexible, and efficient sequencing options. MGI's Genetic Sequencer DNBSEQ-G400 (Fig. 1, MGI, Cat. No. 900-000168-00) high-throughput sequencing platform, utilizes an innovative flow cell system which can support various sequencing modes and an optimized optical and biochemical system that enables the whole sequencing process to be completed within a short period of time, offering the user a simplified and stream-lined sequencing experience. In this application note, we present the evaluation of the performance of MGI's high-throughput sequencing platform, DNBSEQ-G400, compared to two other instruments available on the market, for microbiological sample pooling sequencing using high-throughput sequencing (MGI, Universal Sequencing Reaction Kit - G400 SM FCL PE150, Cat. No. 1000022482*) with ABL's DeepChek® assays and DeepChek® software.

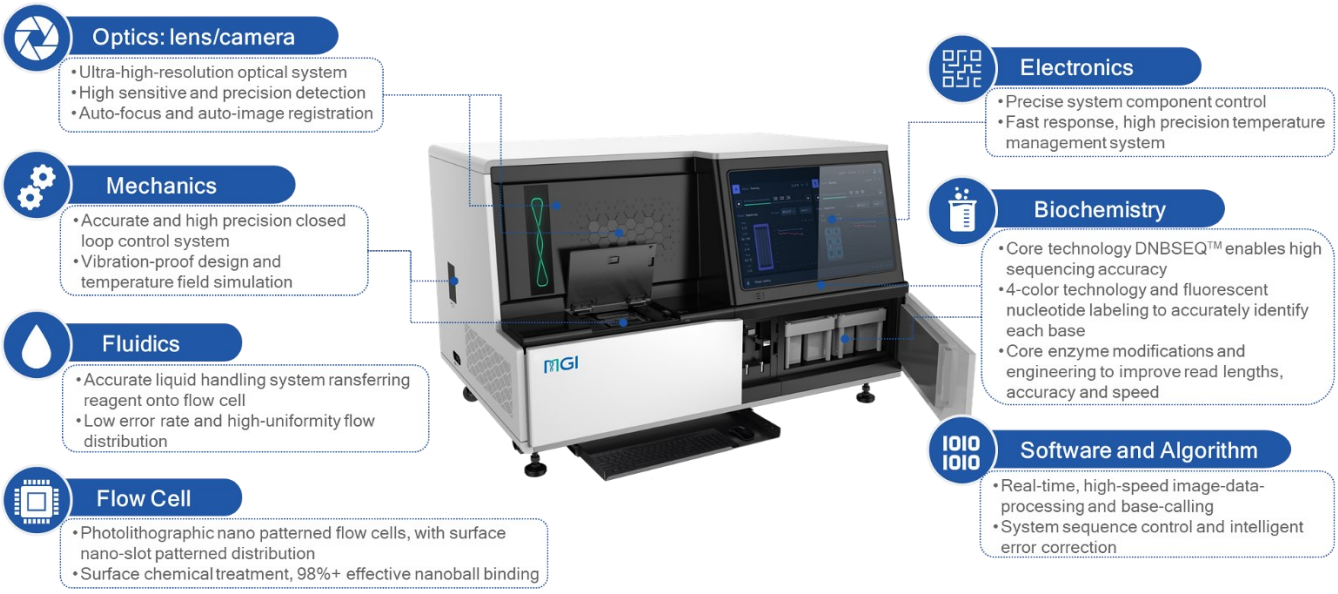

Fig. 1: MGI's DNBSEQ-G400 is built with a new flow cell system that can flexibly support a variety of different sequencing modes. It adopts optimized optical and biochemical systems, which can complete the sequencing process rapidly, providing users with a more streamlined sequencing experience.

Workflow Highlights

PCR/RT-PCR and Library Preparation

To evaluate the microbiological samples pooling sequencing performance of MGI's Genetic Sequencer DNBSEQ-G400, positive leftover plasmas (HIV, HCV and HBV), positive leftover viral transport media (SARS-CoV-2), positive sputum control (Mycobacterium Tuberculosis) and HIV-1 external controls were purified using MagNa Pure24 (Roche). A total of 16 samples were tested: amplifications (Cat. No. ABL: 121A, 122A, 198B, 105A, 107D, 108A, 109A, 184A, 159C, 128A) and libraries (Cat. No. ABL: 116B and 124B) were performed using DeepChek® assays (ABL) intended for target specific and whole genome sequencing and NGS library preparation. The NGS libraries were also converted using the MGIEasy Universal Library Conversion Kit (Cat. No. 1000004155). Libraries were sequenced (2x150bp) using the DNBSEQ-G400 and instrument platforms 1 and 2 for comparison. Output sequences were compared to the interest pathogen reference genomes (Fig. 2).

MGI DNBSEQ™ Sequencing

DNB sequencing takes place on high-density pattern arrays that immobilize individual DNBs for highly parallelized tracking of dNTP incorporation during strand extension. Sequencing starts with the hybridization of DNBs to anchor spots. After primer hybridization, the flow cell is flushed with fluorescently labeled dNTP probes. Unbound probes are washed away, and bound probes are stimulated to fluoresce. High resolution imaging and proprietary algorithms transform signals into high-quality and highly accurate sequencing results.

Sequencing was performed according to the DNBSEQ-G400 protocol employing the PE150 mode. Based on DNBSEQ™ technology (Fig. 3), the DNBSEQ-G400 and compatible DNBSEQ-G400 high throughput sequencing kits generate high quality and highly accurate sequencing results.

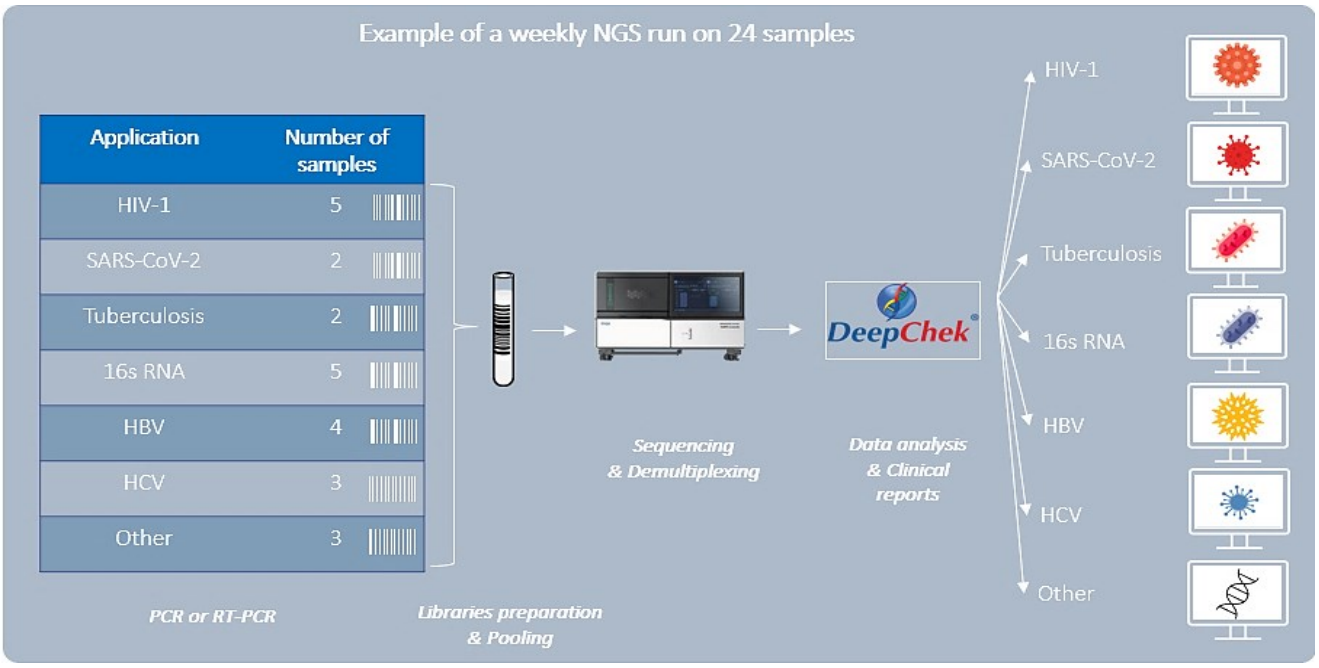

Fig. 2: The workflow of pooling sequencing with DeepChek® assays and MGI sequencing platform. Example of a weekly NGS run on 24 samples.

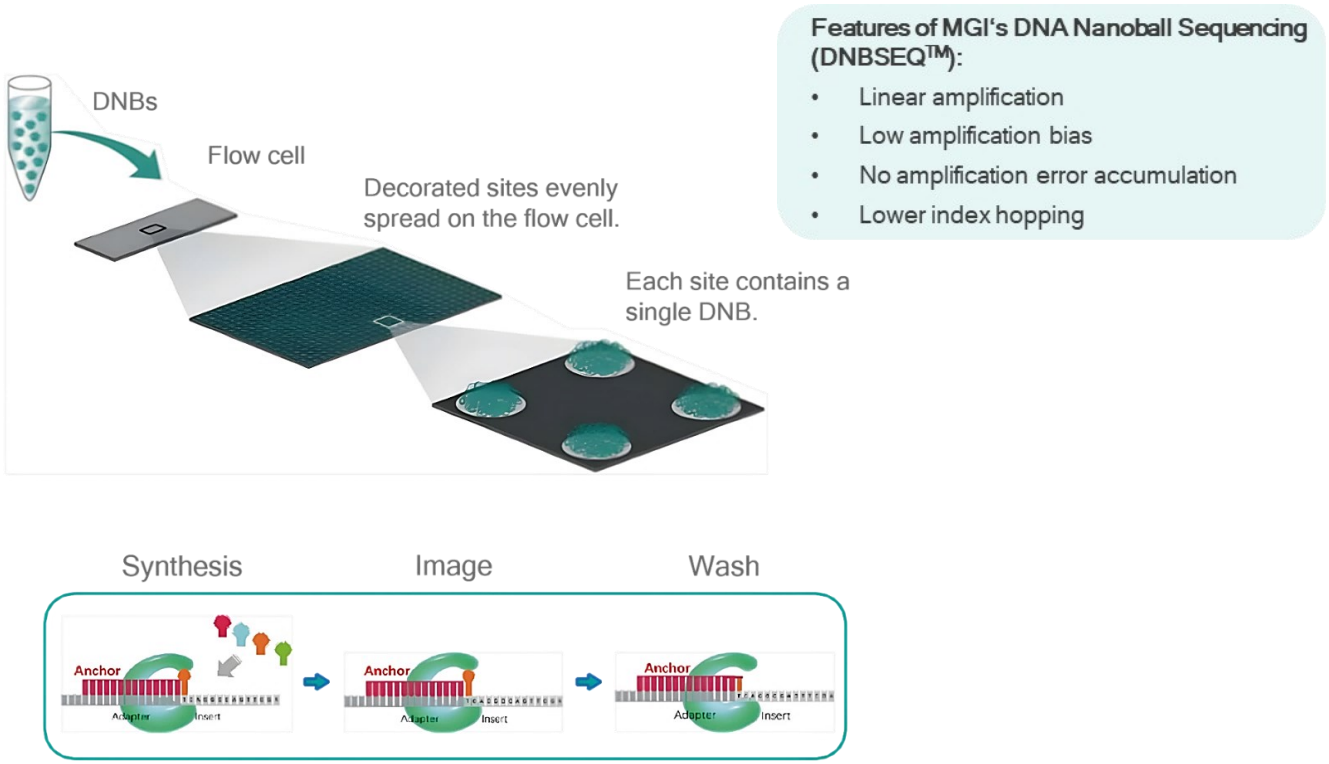

Fig. 3: DNB sequencing uses the combinatory probe anchor system (cPAS) on a patterned array flow cell to provide high sequencing accuracy with improved imaging and reduced index hopping.

Data Analysis and sequencing assembly

The DeepChek® software (ABL, Luxembourg) was used for the analysis of subtypes, mutations and induced drug resistance for all pathogens. DeepChek® (ABL, Luxembourg) is a CE marked downstream software capable of performing automated sequencing analysis from multiple inputs such as Sanger sequencing trace files (ABI), Fasta or FastQ file from NGS workflows (MGI sequencing data). The pipeline consists of 10 major steps which are: 1) Data cleaning, 2) Subtyping, 3) Tropism analysis (for HIV), 4) Alignment, 5) Post-alignment cleaning, 6) Consensus creation, 7) Variant calling, 8) Expert system filtering, 9) Drug resistance calculation and 10) Reporting (Fig. 4). DeepChek® is a secured web application which can be used through cloud access or locally, through pre-configured servers within the IT network of each laboratory. The cloud platform is available on dedicated servers labelled HDS ("Hébergeur de Données de Santé"/Health Data Hosting) which is a certification required for entities such as cloud service providers that host the personal health data governed by European and French laws and collected for delivering preventive, diagnostic, and other health services. Statistical analysis was performed using Prism 9 software (version 9.5.1).

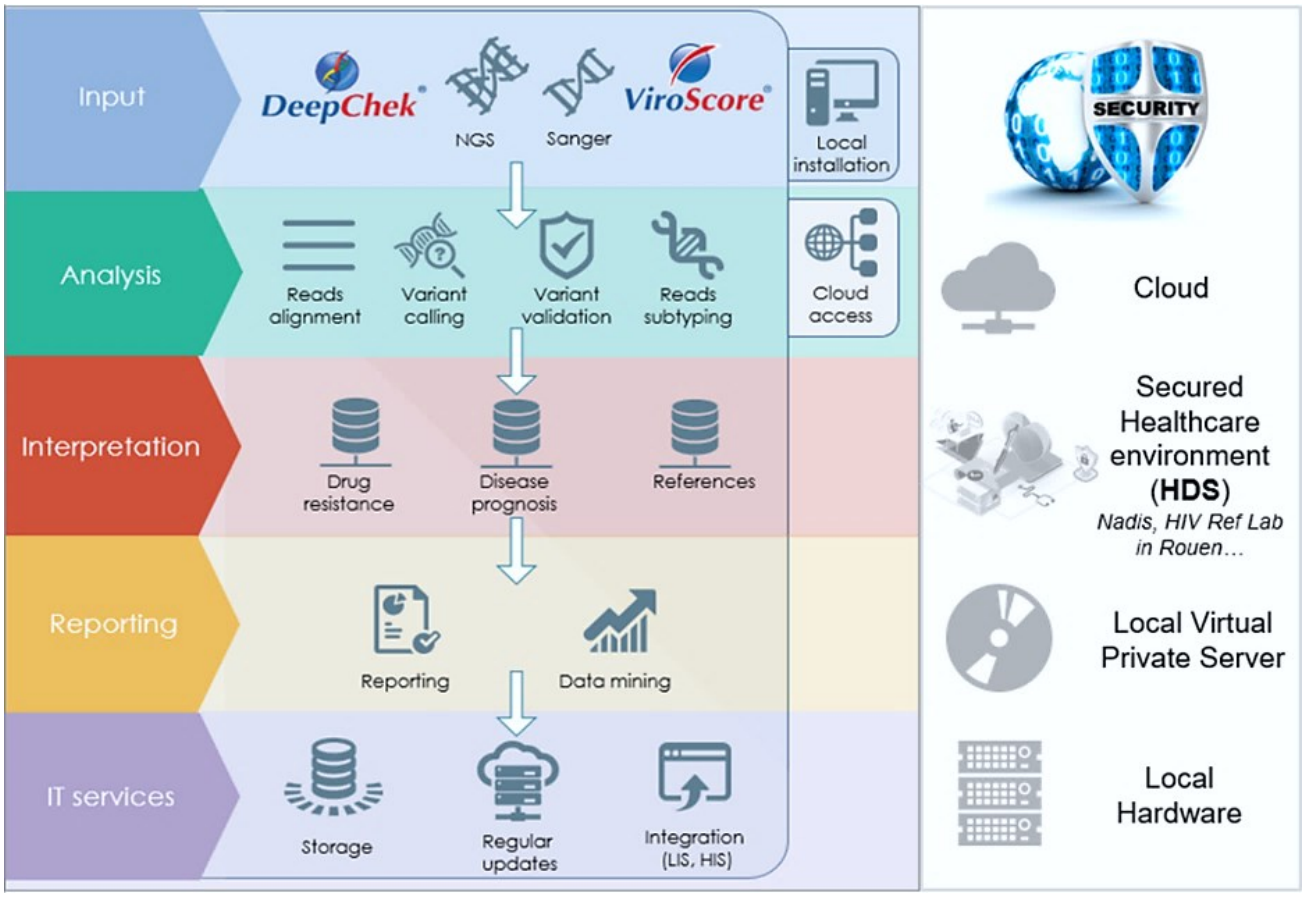

Fig. 4: Data analysis workflow for ABL Diagnostics software systems. The DeepChek® software system is a secured web application which can be used through cloud access or locally, through pre-configured servers. It is made available with regular updates (new clinical databases, guidelines…) and quarterly upgrades (new features, modules, applications…) and can be fully integrated within the IT network of each laboratory (integration with the sequencing platform, with the Laboratory Information System - LIS, with the Hospital Information System - HIS…).

Results

The Q30 score was 95%, and 75% and 86% for DNBSEQ-G400 instrument, and instrument 1 and 2 respectively. The median sequence number per sample was 190.040, and 768.506 and 102.488 for DNBSEQ-G400 instrument and instrument 1 and 2 respectively. Only 0.25% of the DNBSEQ-G400 instrument reads for each sample (randomly from each file and balanced over the 4 lanes) was used. All samples were accurately genotyped, and all mutations of interest were detected with the three NGS platforms: the DNBSEQ-G400 and instrument 1 and 2 (Fig. 5). Significant difference is observed for the percentage of reads mapped to the pathogen between DNBSEQ-G400/instrument 1 and DNBSEQ-G400/instrument 2, ($p < 0.01$ $p = 0.03$), respectively (Fig. 6). No significant difference is observed with the total number of mutations of interest (Fig. 7 and Fig. 8).

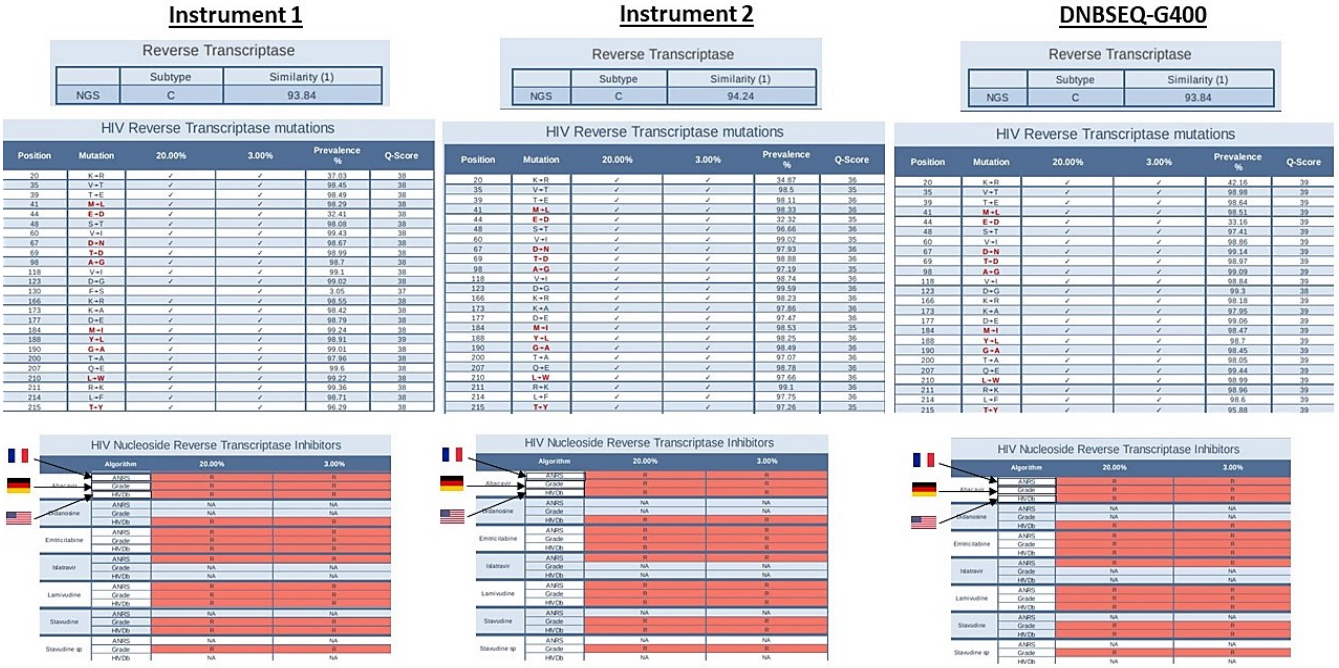

Fig 5: Comparison of the HIV-1 subtype and drug resistance mutation using DeepChek® software.

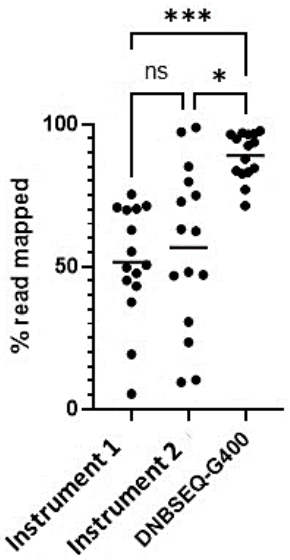

Fig. 6: % of read mapped to the pathogen

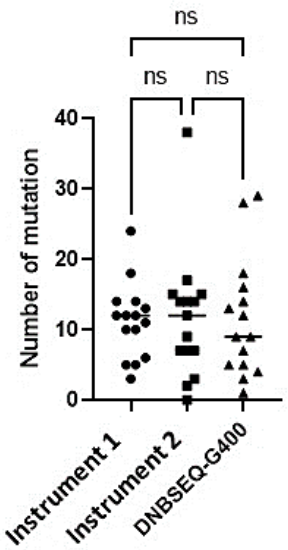

Fig. 7: TOTAL Minority Mutations: (>1% to <20%)

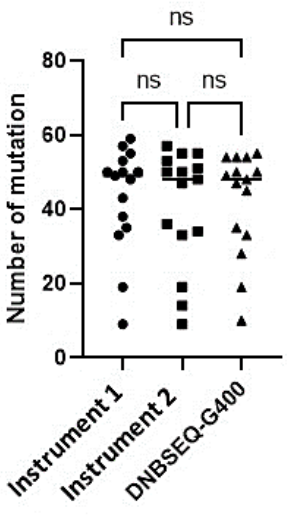

Fig. 8: TOTAL Majority Mutations (>20% to <100%)

Conclusion

Equivalent results between the DNBSEQ-G400 and instrument 1 and instrument 2 were observed for all pathogens. MGI data generation, MGI % of reads and MGI Q30 on the DNBSEQ-G400 were superior to instrument 1 and instrument 2. Next generation sequencing should occupy a major place in microbiology applications testing for subtyping, mutation determination and analysis, and drug resistance surveillance. It should enable to reveal resistant minority variants or new mutations and study their impact. The used sequencing methods show an overall comparable quality: further head-to-head comparisons shall be conducted to better determine the use-case of each platform, turn-around time, and economics. DeepChek® assays using MGI DNBSEQ™ NGS technology with an easy-to-use software, such as the DeepChek® software, has the capacity to accommodate a greater number of samples than those presented in this study and can be used with the G99 and E25 (data not showed: data can be made available upon request).

Ordering information

Product Name	Catalog Number
DeepChek® Assay PROTEASE / REVERSE TRANSCRIPTASE Genotyping and Drug Resistance	121A24
DeepChek® Assay INTEGRASE Genotyping and Drug Resistance	122A24
DeepChek® Assay HIV-1 Full PR/RT/INT Drug Resistance	198B24
DeepChek® Assay Whole Genome HIV-1 Genotyping	170B24
DeepChek® Assay NS5A Genotyping and Drug Resistance	105A24
DeepChek® Assay NS5B Genotyping and Drug Resistance	107D24
DeepChek® Assay NS3 Genotyping and Drug Resistance	108A24
DeepChek® Assay CORE Genotyping	109A24
DeepChek® Assay Whole Genome HBV Genotyping	184A24
DeepChek® Whole Genome SARS-CoV-2 Genotyping	159D24
DeepChek® Assay 13-Plex KB Drug Susceptibility	128A24
DeepChek® Assay UL54 / UL56 / UL97 Drug Resistance	117B24

DeepChek® Assay Whole Genome BKV Genotyping	189A24
DeepChek® Assay Whole Genome HDV Genotyping	199A24
DeepChek® Assay TK / POL Drug Resistance	119A24
DeepChek® Assay Whole Genome Influenza A/B Genotyping	193A24
DeepChek® Assay Whole Genome RSV Genotyping	201A24
DeepChek® NGS Library Preparation + Adapters	116BX - 124BX and other references available if needed for UDI
DeepChek® NGS Clean-up beads	N411-0X (various models)
DeepChek® Software	S-12-023 (various modules including DeepChek®-HIV (S-12-023 (HM))
MicrobioChek® - Software	S-19-MBCK (various modules)
MGIEasy Universal Library Conversion Kit	1000004155
Genetic Sequencer DNBSEQ-G400	900-000168-00
Universal Sequencing Reaction Kit - G400 SM FCL PE150*	1000022482

Disclaimer: The products listed in this document are to be used by trained personnel only. All products are to be used only in accordance with local laws, regulations, and package instructions. For Research Use Only (RUO): not for use in diagnostic procedures, no claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease. Please contact our support team to request the appropriate Instructions for Use (IFU) and registration status of the above-mentioned products for your respective territories. DeepChek® is a registered trademark of Advanced Biological Laboratories (ABL) SA. All other product names, trademarks, and logos are the property of their respective owners.

*Unless otherwise informed, this StandardMPS sequencing reagent is not available in Germany, UK, Sweden, and Switzerland

About MGI

MGI Tech Co., Ltd. (referred to as “MGI”) is committed to building core tools and technologies to lead life science through intelligent innovation. MGI focuses on R&D, production and sales of DNA sequencing instruments, reagents, and related products to support life science research, agriculture, precision medicine and healthcare. MGI is a leading producer of clinical high-throughput gene sequencers, and its multi-omics platforms include genetic sequencing, medical imaging, and laboratory automation.

MGI, founded in 2016 in Shenzhen, China, is one of the few companies worldwide that can develop and mass-produce clinical genetic sequencers. Providing real-time, comprehensive, life-long solutions, its vision is to lead life science innovation.

At present, MGI has more than 1,800 employees and operates in more than 70 countries and regions Wseurvhianng, more than 1,100 customers around the world, with 8 international subsidiaries including Qingdao, Changchun, Kunshan and Hongkong, China, Japan, UAE, Latvia, and USA.

About ABL Diagnostics

ABL DIAGNOSTICS S.A. (ABLD) is a worldwide leading international company offering innovative and proprietary molecular biology assays and end-to-end solutions intended to be used for molecular detection by Polymerase Chain Reaction (PCR) - UltraGene® and for genotyping through DNA sequencing - DeepChek®.

These molecular biology products cover one of the largest portfolio of microbiology applications, growing fast year after year to stick to the market needs, with a primary focus on HIV (with target-specific assays covering all relevant genes used for drug resistance assessment like reverse transcriptase, protease, integrase and with the disruptive Whole Genome Kit), on SARS-CoV-2, on Tuberculosis (with a multiplex assay targeting genes relevant for first line, second line and new-drugs resistance determination), on viral hepatitis B and C, 16s/18s RNA for taxonomy and microbiome analyses and other viral and bacterial targets.

ABL Diagnostics also develops, manufactures, and markets kits for clinical specimen collection - MediaChek® and digital solutions like Nadis®, an Electronic Medical Record (EMR) system used in France in more than 200 hospitals managing patients infected by HIV or Viral Hepatitis.

ABL Diagnostics, based in Woippy, is a public company listed in compartment B of Euronext’s regulated market in Paris (Euronext: ABLD - ISIN: FR001400AHX6). For further information, please visit www.abldiagnostics.com.

ABL Diagnostics
72C Route de Thionville,
57140 WOIPPY, France
abldiagnostics.com
contact@abldiagnostics.com
Tel.: +33 (0)7 83 64 68 50

MGI Tech Co., Ltd.
Building 11, Beishan Industrial Zone,
Yantian District, Shenzhen, CHINA,18083
en.mgi-tech.com
MGI-service@mgi-tech.com
Tel.: +86 4000 966 988